

96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula V have the same molecular weight; and, more preferably, the mixture of compounds of Formula V is a monodispersed mixture.

Q is a halide, preferably chloride or fluoride.

$\text{CH}_3\text{S}(\text{O}_2)\text{Q}$ is methanesulfonyl halide. The methanesulfonyl halide is preferably methanesulfonyl chloride or methanesulfonyl fluoride. More preferably, the methanesulfonyl halide is methanesulfonyl chloride.

The molar ratio of the methane sulfonyl halide to the compound of Formula V is preferably greater than about 1:1, and is more preferably at least about 2:1. By providing an excess of the methane sulfonyl halide, it is assured that substantially all of the compounds of Formula V are reacted to provide the compounds of Formula II. Thus, separation difficulties, which may occur if both compounds of Formula V and compounds of Formula II were present in the reaction product mixture, may be avoided.

Reaction 3 is preferably performed between about -10°C and about 40°C , is more preferably performed between about 0°C and about 35°C , and is most preferably performed between about 0°C and room temperature (approximately 25°C).

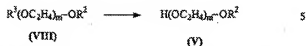
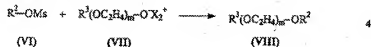
Reaction 3 may be performed for various periods of time as will be understood by those skilled in the art. Reaction 3 is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction 3 is preferably carried out in the presence of an aliphatic amine including, but not limited to, monomethylamine, dimethylamine, trimethylamine, monoethylamine, diethylamine, triethylamine, monoisopropylamine, diisopropylamine, mono-n-butylamine, di-n-butylamine, tri-n-butylamine, monocyclohexylamine, dicyclohexylamine, or mixtures thereof. More preferably, the aliphatic amine is a tertiary amine such as triethylamine.

As will be understood by those skilled in the art, various substantially monodispersed mixtures of compounds of Formula V are commercially available. For example, when R^2 is H or methyl, the compounds of Formula V are PEG or mPEG compounds, respectively, which are commercially available from Aldrich of Milwaukee, Wisconsin; Fluka of Switzerland, and/or TCI America of Portland, Oregon.

When R^2 is a lipophilic moiety such as, for example, higher alkyl, fatty acid, an ester of a fatty acid, cholesteryl, or adamantyl, the compounds of Formula V may be provided by

various methods as will be understood by those skilled in the art. The compounds of Formula V are preferably provided as follows:



R² is a lipophilic moiety, preferably higher alkyl, fatty acid ester, cholesteryl, or adamantyl, more preferably a lower alkyl ester of a fatty acid, and most preferably an ethyl ester of a fatty acid having from 1 to 18 carbon atoms.

R³ is H, benzyl, trityl, tetrahydropyran, or other alcohol protecting groups as will be understood by those skilled in the art.

X₁⁺ is a positive ion as described above with respect to X⁺.

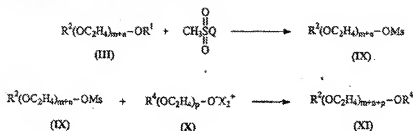
The value of m is as described above.

Regarding reaction 4, a mixture of compounds of Formula VI is reacted with a mixture of compounds of Formula VII under reaction conditions similar to those described above with reference to reaction 1. The mixture of compounds of Formula VI is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula VI have the same molecular weight. More preferably, the mixture of compounds of Formula VI is a monodispersed mixture. The mixture of compounds of Formula VII is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula VII have the same molecular weight. More preferably, the mixture of compounds of Formula VII is a monodispersed mixture.

Regarding reaction 5, the compound of Formula VIII may be hydrolyzed to convert the R² moiety into an alcohol by various methods as will be understood by those skilled in the art. When R³ is benzyl or trityl, the hydrolysis is preferably performed utilizing H₂ in the presence of a palladium-charcoal catalyst as is known by those skilled in the art. Of course, when R³ is H, reaction 5 is unnecessary.

The compound of Formula VI may be commercially available or be provided as described above with reference to reaction 3. The compound of Formula VII may be provided as described above with reference to reaction 2.

Substantially monodispersed mixtures of polymers comprising PEG moieties and having the structure of Formula III above can further be reacted with other substantially monodispersed polymers comprising PEG moieties in order to extend the PEG chain. For example, the following scheme may be employed:



Ms, m and n are as described above with reference to reaction 1; p is similar to n and m, and X_1^+ is similar to X^+ as described above with reference to reaction 1. Q is as described above with reference to reaction 3. R^2 is as described above with reference to reaction 1 and is preferably lower alkyl. R^1 is H. Reaction 6 is preferably performed in a manner similar to that described above with reference to reaction 3. Reaction 7 is preferably performed in a manner similar to that described above with reference to reaction 1. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula III have the same molecular weight, and, more preferably, the mixture of compounds of Formula III is a monodispersed mixture. The mixture of compounds of Formula X is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula X have the same molecular weight, and, more preferably, the mixture of compounds of Formula X is a monodispersed mixture.

A process according to embodiments of the present invention is illustrated by the scheme shown in Figure 1, which will now be described. The synthesis of substantially monodispersed polyethylene glycol-containing oligomers begins by the preparation of the monobenzyl ether (I) of a substantially monodispersed polyethylene glycol. An excess of a commercially available substantially monodispersed polyethylene glycol is reacted with benzyl chloride in the presence of aqueous sodium hydroxide as described by Coudert *et al*

(*Synthetic Communications*, 16(1): 19-26 (1986)). The sodium salt of 1 is then prepared by the addition of NaH, and this sodium salt is allowed to react with the mesylate synthesized from the ester of a hydroxyalkanoic acid (2). The product (3) of the displacement of the mesylate is debenzylated via catalytic hydrogenation to obtain the alcohol (4). The mesylate (5) of this alcohol may be prepared by addition of methanesulfonyl chloride and used as the electrophile in the reaction with the sodium salt of the monomethyl ether of a substantially monodispersed polyethylene glycol derivative, thereby extending the polyethylene glycol portion of the oligomer to the desired length, obtaining the elongated ester (6). The ester may be hydrolyzed to the acid (7) in aqueous base and transformed into the activated ester (8) by reaction with a carbodiimide and N-hydroxysuccinimide. While the oligomer illustrated in Figure 1 is activated using N-hydroxysuccinimide, it is to be understood that various other reagents may be used to activate oligomers of the present invention including, but not limited to, active phenyl chloroformates such as *para*-nitrophenyl chloroformate, phenyl chloroformate, 3,4-phenyldichloroformate, and 3,4-phenyldichloroformate; trisylation; and acetal formation.

Still referring to Figure 1, q is from 1 to 24. Preferably, q is from 1 to 18, and q is more preferably from 4 to 16. R¹ is a moiety capable of undergoing hydrolysis to provide the carboxylic acid. R² is preferably lower alkyl and is more preferably ethyl. The variables n and m are as described above with reference to reaction 1.

All starting materials used in the procedures described herein are either commercially available or can be prepared by methods known in the art using commercially available starting materials.

The present invention will now be described with reference to the following examples. It should be appreciated that these examples are for the purposes of illustrating aspects of the present invention, and do not limit the scope of the invention as defined by the claims.

EXAMPLES

Examples 1 through 10

Reactions in Examples 1 through 10 were carried out under nitrogen with magnetic stirring, unless otherwise specified. "Work-up" denotes extraction with an organic solvent, washing of the organic phase with saturated NaCl solution, drying (MgSO₄), and evaporation.

(rotary evaporator). Thin layer chromatography was conducted with Merck glass plates precoated with silica gel 60°F - 254 and spots were visualized by iodine vapor. All mass spectra were determined by Macromolecular Resources Colorado State University, CO and are reported in the order m/z , (relative intensity). Elemental analyses and melting points were performed by Galbraith Laboratories, Inc., Knoxville, TN. Examples 1-10 refer to the scheme illustrated in Figure 2.

Example 1

8-Methoxy-1-(methylsulfonyloxy)-3,6-dioxaoctane (9)

A solution of non-polydispersed triethylene glycol monomethyl ether molecules (4.00 mL, 4.19 g, 25.5 mmol) and triethylamine (4.26 mL, 3.09 g, 30.6 mmol) in dry dichloromethane (50 mL) was chilled in an ice bath and placed under a nitrogen atmosphere. A solution of methanesulfonyl chloride (2.37 mL, 3.51 g, 30.6 mmol) in dry dichloromethane (20 mL) was added dropwise from an addition funnel. Ten minutes after the completion of the chloride addition, the reaction mixture was removed from the ice bath and allowed to come to room temperature. The mixture was stirred for an additional hour, at which time TLC (CHCl_3 with 15% MeOH as the eluant) showed no remaining triethylene glycol monomethyl ether.

The reaction mixture was diluted with another 75 mL of dichloromethane and washed successively with saturated NaHCO_3 , water and brine. The organics were dried over Na_2SO_4 , filtered and concentrated in vacuo to give a non-polydispersed mixture of compounds 9 as a clear oil (5.31g, 86%).

Example 2

Ethylene glycol mono methyl ether (10) ($m=4,5,6$)

To a stirred solution of non-polydispersed compound 11 (35.7 mmol) in dry DMF (25.7 mL), under N_2 was added in portion a 60% dispersion of NaH in mineral oil, and the mixture was stirred at room temperature for 1 hour. To this salt 12 was added a solution of non-polydispersed mesylate 9 (23.36) in dry DMF (4 ml) in a single portion, and the mixture was stirred at room temperature for 3.5 hours. Progress of the reaction was monitored by TLC (12% $\text{CH}_3\text{OH}-\text{CHCl}_3$). The reaction mixture was diluted with an equal amount of 1N HCl, and extracted with ethyl acetate (2 x 20 mL) and discarded. Extraction of aqueous

solution and work-up gave non-polydispersed polymer 10 (82-84% yield).

Example 3

3,6,9,12,15,18,21-Heptaeradecosan-1-ol (10) (m=4)

Oil; Rf 0.46 (methanol : chloroform = 3:22); MS m/z calc'd for $C_{19}H_{38}O_2$ 340.21 ($M^+ + 1$), found 341.2.

Example 4

3,6,9,12,15,18,21,24-Octaerapentacosan-1-ol (10) (m=5)

Oil; Rf 0.43 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{27}H_{54}O_2$ 384.24 ($M^+ + 1$), found 385.3.

Example 5

3,6,9,12,15,18,21,24,27-Nonaxaeractacosan-1-ol (10) (m=6)

Oil; Rf 0.42 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{29}H_{58}O_2$ 428.26 ($M^+ + 1$), found 429.3.

Example 6

20-methoxy-1-(methylsulfonyl)oxy-3,6,9,12,15,18-hexaeracosane (14)

Non-polydispersed compound 14 was obtained in quantitative yield from the alcohol 13 (m=4) and methanesulfonyl chloride as described for 9, as an oil; Rf 0.4 (ethyl acetate : acetonitrile = 1:5); MS m/z calc'd for $C_{27}H_{54}O_4$ 433.21 ($M^+ + 1$), found 433.469.

Example 7

Ethylene glycol mono methyl ether (15) (m=3,4,5)

The non-polydispersed compounds 15 were prepared from a diol by using the procedure described above for compound 10.

Example 8

3,6,9,12,15,18,21,24,27,30-Decaeraheneicosan-1-ol (15) (m=3)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{31}H_{62}O_2$ 472.29 ($M^+ + 1$), found 472.29.

Example 93,6,9,12,15,18,21,24,27,30,33-Unecaoxotetracosanol (15) ($m=4$)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{23}H_{48}O_{12}$ 516.31 ($M^+ + 1$), found 516.31.

Example 103,6,9,12,15,18,21,24,27,30,33,36-Dodecaoxaheptacosanol (15) ($m=5$)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{23}H_{48}O_{13}$ 560.67 ($M^+ + 1$), found 560.67.

Examples 11 through 18 refer to the scheme illustrated in Figure 3.

Example 11

Hexaethylene glycol monobenzyl ether (16)

An aqueous sodium hydroxide solution prepared by dissolving 3.99 g (100 mmol) NaOH in 4 ml water was added slowly to non-polydispersed hexaethylene glycol (28.175 g, 25 ml, 100 mmol). Benzyl chloride (3.9 g, 30.8 mmol, 3.54 ml) was added and the reaction mixture was heated with stirring to 100°C for 18 hours. The reaction mixture was then cooled, diluted with brine (250 ml) and extracted with methylene chloride (200 ml x 2). The combined organic layers were washed with brine once, dried over Na_2SO_4 , filtered and concentrated in vacuo to a dark brown oil. The crude product mixture was purified via flash chromatography (silica gel, gradient elution: ethyl acetate to 9/1 ethyl acetate/methanol) to yield 8.099 g (70 %) of non-polydispersed 16 as a yellow oil.

Example 12

Ethyl 6-methylsulfonyloxyhexanoate (17)

A solution of non-polydispersed ethyl 6-hydroxyhexanoate (50.76 ml, 50.41 g, 227 mmol) in dry dichloromethane (75 ml) was chilled in a ice bath and placed under a nitrogen atmosphere. Triethylamine (34.43 ml, 24.99 g, 247 mmol) was added. A solution of methanesulfonyl chloride (19.15 ml, 28.3 g, 247 mmol) in dry dichloromethane (75 ml) was added dropwise from an addition funnel. The mixture was stirred for three and one half

hours, slowly being allowed to come to room temperature as the ice bath melted. The mixture was filtered through silica gel, and the filtrate was washed successively with water, saturated NaHCO_3 , water and brine. The organics were dried over Na_2SO_4 , filtered and concentrated in vacuo to a pale yellow oil. Final purification of the crude product was achieved by flash chromatography (silica gel, 1/1 hexanes/ethyl acetate) to give the non-polydispersed product (46.13 g, 85 %) as a clear, colorless oil. FAB MS: m/e 239 ($M+H$), 193 ($M-C_2H_5O$).

Example 13

6-[2-[2-(2-[2-(2-Benzyloxyethoxy)ethoxy]ethoxy)-ethoxy]-ethoxy]-hexanoic acid ethyl ester (18)

Sodium hydride (3.225 g or a 60 % oil dispersion, 80.6 mmol) was suspended in 80 ml of anhydrous toluene, placed under a nitrogen atmosphere and cooled in an ice bath. A solution of the non-polydispersed alcohol 16 (27.3 g, 73.3 mmol) in 80 ml dry toluene was added to the NaH suspension. The mixture was stirred at 0°C for thirty minutes, allowed to come to room temperature and stirred for another five hours, during which time the mixture became a clear brown solution. The non-polydispersed mesylate 17 (19.21 g, 80.6 mmol) in 80 ml dry toluene was added to the NaH/alcohol mixture, and the combined solutions were stirred at room temperature for three days. The reaction mixture was quenched with 50 ml methanol and filtered through basic alumina. The filtrate was concentrated in vacuo and purified by flash chromatography (silica gel, gradient elution: 3/1 ethyl acetate/hexanes to ethyl acetate) to yield the non-polydispersed product as a pale yellow oil (16.52 g, 44 %). FAB MS: m/e 515 ($M+H$).

Example 14

6-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]-ethoxy]-ethoxy]-hexanoic acid ethyl ester (19)

Non-polydispersed benzyl ether 18 (1.03 g, 2.0 mmol) was dissolved in 25 ml ethanol. To this solution was added 270 mg 10 % Pd/C, and the mixture was placed under a hydrogen atmosphere and stirred for four hours, at which time TLC showed the complete disappearance of the starting material. The reaction mixture was filtered through Celite 545 to remove the catalyst, and the filtrate was concentrated in vacuo to yield the non-

polydispersed title compound as a clear oil (0.67 g, 79 %). FAB MS: *m/e* 425 (M+H), 447 (M+Na).

Example 15

6-{2-[2-(2-[2-(2-methylsulfonylethoxy)ethoxy]ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (20)

The non-polydispersed alcohol 19 (0.835 g, 1.97 mmol) was dissolved in 3.5 ml dry dichloromethane and placed under a nitrogen atmosphere. Triethylamine (0.301 ml, 0.219 g, 2.16 mmol) was added and the mixture was chilled in an ice bath. After two minutes, the methanesulfonyl chloride (0.16 ml, 0.248 g, 2.16 mmol) was added. The mixture was stirred for 15 minutes at 0 °C, then at room temperature for two hours. The reaction mixture was filtered through silica gel to remove the triethylammonium chloride, and the filtrate was washed successively with water, saturated NaHCO₃, water and brine. The organics were dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 9/1 ethyl acetate/methanol) to give non-polydispersed compound 20 as a clear oil (0.819 g, 83 %). FAB MS: *m/e* 503 (M+H).

Example 16

6-{2-[1-[2-(2-[2-(2-methoxyethoxy)ethoxy]-ethoxy)-ethoxy]-ethoxy]-ethoxy}-hexanoic acid ethyl ester (21)

NaH (88 mg of a 60 % dispersion in oil, 2.2 mmol) was suspended in anhydrous toluene (3 ml) under N₂ and chilled to 0 °C. Non-polydispersed diethylene glycol monomethyl ether (0.26 ml, 0.26 g, 2.2 mmol) that had been dried via azeotropic distillation with toluene was added. The reaction mixture was allowed to warm to room temperature and stirred for four hours, during which time the cloudy grey suspension became clear and yellow and then turned brown. Mesylate 20 (0.50 g, 1.0 mmol) in 2.5 ml dry toluene was added. After stirring at room temperature over night, the reaction was quenched by the addition of 2 ml of methanol and the resultant solution was filtered through silica gel. The filtrate was concentrated in vacuo and the FAB MS: *m/e* 499 (M+H), 521 (M+Na). Additional purification by preparatory chromatography (silica gel, 19/3 chloroform/methanol) provided the non-polydispersed product as a clear yellow oil (0.302 g 57 %). FAB MS: *m/e* 527 (M+H), 549 (M+Na).

Example 17**6-(2-[2-[2-(2-[2-(2-methoxyethoxy)ethoxy]-ethoxy)-ethoxy]-ethoxy]-hexanoic acid (22)**

Non-polydispersed ester 21 (0.25 g, 0.46 mmol) was stirred for 18 hours in 0.71 ml of 1 N NaOH. After 18 hours, the mixture was concentrated in vacuo to remove the alcohol and the residue dissolved in a further 10 ml of water. The aqueous solution was acidified to pH 2 with 2 N HCl and the product was extracted into dichloromethane (30 ml x 2). The combined organics were then washed with brine (25 ml x 2), dried over Na₂SO₄, filtered and concentrated in vacuo to yield the non-polydispersed title compound as a yellow oil (0.147 g, 62 %). FAB MS: *m/e* 499 (M+H), 521 (M+Na).

Example 18**6-(2-[2-[2-(2-[2-(2-methoxyethoxy)ethoxy]-ethoxy)-ethoxy]-ethoxy]-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (23)**

Non-polydispersed acid 22 (0.209 g, 0.42 mmol) were dissolved in 4 ml of dry dichloromethane and added to a dry flask already containing NHS (N-hydroxysuccinimide) (37.8 mg, 0.502 mmol) and EDC (1-[3-dimethylaminopropyl]-3-ethylcarbodiimide hydrochloride) (98.0 mg, 0.502 mmol) under a N₂ atmosphere. The solution was stirred at room temperature overnight and filtered through silica gel to remove excess reagents and the urea formed from the EDC. The filtrate was concentrated in vacuo to provide the non-polydispersed product as a dark yellow oil (0.235 g, 94 %). FAB MS: *m/e* 596 (M+H), 618 (M+Na).

Examples 19 through 24 refer to the scheme illustrated in Figure 4.

Example 19**Mesylate of triethylene glycol monomethyl ether (24)**

To a solution of CH₂Cl₂ (100 mL) cooled to 0°C in an ice bath was added non-polydispersed triethylene glycol monomethyl ether (25 g, 0.15 mol). Then triethylamine (29.5 mL, 0.22 mol) was added and the solution was stirred for 15 min at 0°C, which was followed by dropwise addition of methanesulfonyl chloride (13.8 mL, 0.18 mol, dissolved in

20 mL CH_2Cl_2). The reaction mixture was stirred for 30 min at 0°C , allowed to warm to room temperature, and then stirred for 2 h. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 ~200 mL), then washed with H_2O (300 mL), 5% NaHCO_3 (300 mL), H_2O (300 mL), sat. NaCl (300 mL), dried MgSO_4 , and evaporated to dryness. The oil was then placed on a vacuum line for ~2h to ensure dryness and afforded the non-polydispersed title compound as a yellow oil (29.15 g, 80% yield).

Example 20

Heptaethylene glycol monomethyl ether (25)

To a solution of non-polydispersed tetraethylene glycol (51.5 g, 0.27 mol) in THF (1L) was added potassium *t*-butoxide (14.8 g, 0.13 mol, small portions over ~30 min). The reaction mixture was then stirred for 1h and then 24 (29.15 g, 0.12 mol) dissolved in THF (90 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , ~200 mL) and evaporated to dryness. The oil was then dissolved in HCl (250 mL, 1 N) and washed with ethyl acetate (250 mL) to remove excess 24. Additional washings of ethyl acetate (125 mL) may be required to remove remaining 24. The aqueous phase was washed repetitively with CH_2Cl_2 (125 mL volumes) until most of the 25 has been removed from the aqueous phase. The first extraction will contain 24, 25, and decoupled side product and should be back extracted with HCl (125 mL, 1N). The organic layers were combined and evaporated to dryness. The resultant oil was then dissolved in CH_2Cl_2 (100 mL) and washed repetitively with H_2O (50 mL volumes) until 25 was removed. The aqueous fractions were combined, total volume 500 mL, and NaCl was added until the solution became cloudy and then was washed with CH_2Cl_2 (2 x 500 mL). The organic layers were combined, dried MgSO_4 , and evaporated to dryness to afford a the non-polydispersed title compound as an oil (16.9 g, 41% yield). It may be desirable to repeat one or more steps of the purification procedure to ensure high purity.

Example 21

8-Bromooctanoate (26)

To a solution of 8-bromooctanoic acid (5.0 g, 22 mmol) in ethanol (100 mL) was added H_2SO_4 (0.36 mL, 7.5 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat.

NaHCO_3 (2 x 100 mL), H_2O (100 mL), dried MgSO_4 , and evaporated to dryness to afford a clear oil (5.5 g, 98% yield).

Example 22

Synthesis of MPEG7-C8 ester (27)

To a solution of the non-polydispersed compound 25 (3.0 g, 8.8 mmol) in ether (90 mL) was added potassium *t*-butoxide (1.2 g, 9.5 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of the non-polydispersed compound 26 (2.4 g, 9.6 mmol), dissolved in ether (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried MgSO_4 , and evaporated to dryness. Column chromatography (Silica, ethyl acetate to ethyl acetate/methanol, 10:1) was performed and afforded the non-polydispersed title compound as a clear oil (0.843 g, 19% yield).

Example 23

MPEG7-C8 acid (28)

To the oil of the non-polydispersed compound 27 (0.70 g, 1.4 mmol) was added 1N NaOH (2.0 mL) and the reaction mixture was stirred for 4h. The crude reaction mixture was concentrated, acidified (pH=2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The organic layers were combined, washed sat. NaCl, dried MgSO_4 , and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (0.35 g, 33% yield).

Example 24

Activation of MPEG7-C8 acid (29)

Non-polydispersed mPEG7-C8-acid 28 (0.31g, 0.64 mmol) was dissolved in 3 ml of anhydrous methylene chloride and then solution of N-hydroxysuccinimide (0.079g, 0.69 mmol) and EDCT-HCl (135.6 mg, 0.71 mmol) in anhydrous methylene chloride added. Reaction was stirred for several hours, then washed with 1N HCl, water, dried over MgSO_4 , filtered and concentrated. Crude material was purified by column chromatography, concentrated to afford the non-polydispersed title compound as a clear oil and dried *via* vacuum.

Examples 25 through 29 refer to the scheme illustrated in Figure 5.

Example 25

10-hydroxydecanoate (30)

To a solution of non-polydispersed 10-hydroxydecanoic acid (5.0 g, 25.5 mmol) in ethanol (100 mL) was added H_2SO_4 (0.43 mL, 8.8 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. NaHCO_3 (2 x 100 mL), H_2O (100 mL), dried MgSO_4 , and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (5.9 g, 98% yield).

Example 26

Mesylate of 10-hydroxydecanoate (31)

To a solution of CH_2Cl_2 (27 mL) was added non-polydispersed 10-hydroxydecanoate 30 (5.6 g, 26 mmol) and cooled to 0°C in an ice bath. Then triethylamine (5 mL, 37 mmol) was added and the reaction mixture was stirred for 15 min at 0°C . Then methanesulfonyl chloride (2.7 mL, 24 mmol) dissolved in CH_2Cl_2 (3 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , 80 mL) and the filtrate was washed H_2O (100 mL), 5% NaHCO_3 (2 x 100 mL), H_2O (100 mL), sat. NaCl (100 mL), dried MgSO_4 , and evaporated to dryness to afford the non-polydispersed title compound as a yellowish oil (7.42 g, 97% yield).

Example 27

MPEG₇₋₁₀ Ester (32)

To a solution of non-polydispersed heptaethylene glycol monomethyl ether 25 (2.5 g, 7.3 mmol) in tetrahydrofuran (100 mL) was added sodium hydride (0.194 g, 8.1 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of mesylate of non-polydispersed 10-hydroxydecanoate 31 (2.4 g, 8.1 mmol), dissolved in tetrahydrofuran (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , ~200 mL) and evaporated to dryness. The

resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried $MgSO_4$, evaporated to dryness, chromatographed (silica, ethyl acetate/methanol, 10:1), and chromatographed (silica, ethyl acetate) to afford the non-polydispersed title compound as a clear oil (0.570 g, 15% yield).

Example 28

MPEG₇-C₁₈ Acid (33)

To the oil of non-polydispersed mPEG₇-C₁₈ ester 32 (0.570 g, 1.1 mmol) was added 1N NaOH (1.6 mL) and the reaction mixture was stirred overnight. The crude reaction mixture was concentrated, acidified (pH=2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The organic layers were combined, washed sat. NaCl (2 x 50 mL), dried $MgSO_4$, and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (0.340 g, 62% yield).

Example 29

Activation of MPEG₇-C₁₈ Acid (34)

The non-polydispersed acid 33 was activated using procedures similar to those described above in Example 24.

Examples 30 and 31 refer to the scheme illustrated in Figure 6.

Example 30

Synthesis of C18(PEG6) Oligomer (36)

Non-polydispersed stearoyl chloride 35 (0.7g, 2.31 mmol) was added slowly to a mixture of PEG6 (5 g, 17.7 mmol) and pyridine (0.97g, 12.4 mmol) in benzene. The reaction mixture was stirred for several hours (~5). The reaction was followed by TLC using ethylacetate/methanol as a developing solvent. Then the reaction mixture was washed with water, dried over $MgSO_4$, concentrated and dried *via* vacuum. Purified non-polydispersed compound 36 was analyzed by FABMS: m/e 349/ M^+H .

Example 31**Activation of C18(PEG6) Oligomer**

Activation of non-polydispersed C18(PEG6) oligomer was accomplished in two steps:

1) Non-polydispersed stearoyl-PEG6 36 (0.8 g, 1.46 mmol) was dissolved in toluene and added to a phosgene solution (10 ml, 20 % in toluene) which was cooled with an ice bath. The reaction mixture was stirred for 1 h at 0°C and then for 3 h at room temperature. Then phosgene and toluene were distilled off and the remaining non-polydispersed stearoyl PEG6 chloroformate 37 was dried over P_2O_5 overnight.

2) To a solution of non-polydispersed stearoyl PEG6 chloroformate 36 (0.78 g, 1.27 mmol) and TEA (128 mg, 1.27 mmol) in anhydrous methylene chloride, N-hydroxy succinimide (NHS) solution in methylene chloride was added. The reaction mixture was stirred for 16 hours, then washed with water, dried over $MgSO_4$, filtered, concentrated and dried *via* vacuum to provide the non-polydispersed activated C18(PEG6) oligomer 38.

Examples 32 through 37 refer to the scheme illustrated in Figure 7.

Example 32**Tetraethylene glycol monobenzylether (39)**

To the oil of non-polydispersed tetraethylene glycol (19.4 g, 0.10 mol) was added a solution of NaOH (4.0 g in 4.0 mL) and the reaction was stirred for 15 min. Then benzyl chloride (3.54 mL, 30.8 mmol) was added and the reaction mixture was heated to 100°C and stirred overnight. The reaction mixture was cooled to room temperature, diluted with sat. NaCl (250 mL), and washed CH_2Cl_2 (2 x 200 mL). The organic layers were combined, washed sat. NaCl, dried $MgSO_4$, and chromatographed (silica, ethyl acetate) to afford the non-polydispersed title compound as a yellow oil (6.21 g, 71% yield).

Example 33**Mesylate of tetraethylene glycol monobenzylether (40)**

To a solution of CH_2Cl_2 (20 mL) was added non-polydispersed tetraethylene glycol monobenzylether 39 (6.21 g, 22 mmol) and cooled to 0°C in an ice bath. Then triethylamine (3.2 mL, 24 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then

methanesulfonyl chloride (1.7 mL, 24 mmol) dissolved in CH_2Cl_2 (2 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 , 80 mL) and the filtrate was washed H_2O (100 mL), 5% NaHCO_3 (2×100 mL), H_2O (100 mL), sat. NaCl (100 mL), and dried MgSO_4 . The resulting yellow oil was chromatographed on a pad of silica containing activated carbon (10 g) to afford the non-polydispersed title compound as a clear oil (7.10 g, 89% yield).

Example 34

Octaethylene glycol monobenzylether (41)

To a solution of tetrahydrofuran (140 mL) containing sodium hydride (6.43 g, 18 mmol) was added dropwise a solution of non-polydispersed tetraethylene glycol (3.5 g, 18 mmol) in tetrahydrofuran (10 mL) and the reaction mixture was stirred for 1 h. Then mesylate of non-polydispersed tetraethylene glycol monobenzylether 40 (6.0 g, 16.5 mmol) dissolved in tetrahydrofuran (10 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed, CH_2Cl_2 , 250 mL) and the filtrate was washed H_2O , dried MgSO_4 , and evaporated to dryness. The resultant oil was chromatographed (silica, ethyl acetate/methanol, 10:1) and chromatographed (silica, chloroform/methanol, 25:1) to afford the non-polydispersed title compound as a clear oil (2.62 g, 34% yield).

Example 35

Synthesis of Stearate PEG8-Benzyl (43)

To a stirred cooled solution of non-polydispersed octaethylene glycol monobenzylether 41 (0.998 g, 2.07 mmol) and pyridine (163.9 mg, 2.07 mmol) was added non-polydispersed stearoyl chloride 42 (627.7 mg, 2.07 mmol) in benzene. The reaction mixture was stirred overnight (18 hours). The next day the reaction mixture was washed with water, dried over MgSO_4 , concentrated and dried via vacuum. Then the crude product was chromatographed on flash silica gel column, using 10% methanol/90% chloroform. The fractions containing the product were combined, concentrated and dried via vacuum to afford the non-polydispersed title compound.

Example 36**Hydrogenolysis of Stearate-PEG8-Benzyl**

To a methanol solution of non-polydispersed stearate-PEG8-Bzl 43 (0.854g 1.138 mmol) Pd/C(10%) (palladium, 10% wt. on activated carbon) was added. The reaction mixture was stirred overnight (18 hours) under hydrogen. Then the solution was filtered, concentrated and purified by flash column chromatography using 10% methanol/90% chloroform, fractions with R_f =0.6 collected, concentrated and dried to provide the non-polydispersed acid 44.

Example 37**Activation of C18(PEG8) Oligomer**

Two step activation of non-polydispersed stearate-PEG8 oligomer was performed as described for stearate-PEG6 in Example 31 above to provide the non-polydispersed activated C18(PEG8) oligomer 45.

Example 38**Synthesis of Activated Triethylene Glycol Monomethyl Oligomers**

The following description refers to the scheme illustrated in Figure 8. A solution of toluene containing 20% phosgene (100 ml, approximately 18.7 g, 189 mmol phosgene) was chilled to 0°C under a N_2 atmosphere. Non-polydispersed mTEG (triethylene glycol, monomethyl ether, 7.8 g, 47.5 mmol) was dissolved in 25 mL anhydrous ethyl acetate and added to the chilled phosgene solution. The mixture was stirred for one hour at 0°C, then allowed to warm to room temperature and stirred for another two and one half hours. The remaining phosgene, ethyl acetate and toluene were removed via vacuum distillation to leave the non-polydispersed mTEG chloroformate 46 as a clear oily residue.

The non-polydispersed residue 46 was dissolved in 50 mL of dry dichloromethane to which was added TEA (triethylamine, 6.62 mL, 47.5 mmol) and NHS (N-hydroxysuccinimide, 5.8 g, 50.4 mmol). The mixture was stirred at room temperature under a dry atmosphere for twenty hours during which time a large amount of white precipitate appeared. The mixture was filtered to remove this precipitate and concentrated *in vacuo*. The resultant oil 47 was taken up in dichloromethane and washed twice with cold deionized water, twice with 1N HCl and once with brine. The organics were dried over

MgSO₄, filtered and concentrated to provide the non-polydispersed title compound as a clear, light yellow oil. If necessary, the NHS ester could be further purified by flash chromatography on silica gel using EtOAc as the eluant.

Example 39

Synthesis of Activated Palmitate-TEG Oligomers

The following description refers to the scheme illustrated in Figure 9. Non-polydispersed palmitic anhydride (5 g, 10 mmol) was dissolved in dry THF (20 mL) and stirred at room temperature. To the stirring solution, 3 mol excess of pyridine was added followed by non-polydispersed triethylene glycol (1.4 mL). The reaction mixture was stirred for 1 hour (progress of the reaction was monitored by TLC; ethyl acetate-chloroform; 3:7). At the end of the reaction, THF was removed and the product was mixed with 10% H₂SO₄ acid and extracted ethyl acetate (3 x 30 mL). The combined extract was washed sequentially with water, brine, dried over MgSO₄, and evaporated to give non-polydispersed product 48. A solution of N,N'-disuccinimidyl carbonate (3 mmol) in DMF (~10 mL) is added to a solution of the non-polydispersed product 48 (1 mmol) in 10 mL of anhydrous DMF while stirring. Sodium hydride (3 mmol) is added slowly to the reaction mixture. The reaction mixture is stirred for several hours (e.g., 5 hours). Diethyl ether is added to precipitate the activated oligomer. This process is repeated 3 times and the product is finally dried.

Example 40

Synthesis of Activated Hexaethylene Glycol Monomethyl Oligomers

The following description refers to the scheme illustrated in Figure 10. Non-polydispersed activated hexaethylene glycol monomethyl ether was prepared analogously to that of non-polydispersed triethylene glycol in Example 39 above. A 20% phosgene in toluene solution (35 mL, 6.66 g, 67.4 mmol phosgene) was chilled under a N₂ atmosphere in an ice/salt water bath. Non-polydispersed hexaethylene glycol 50 (1.85 mL, 2.0 g, 6.74 mmol) was dissolved in 5 mL anhydrous EtOAc and added to the phosgene solution via syringe. The reaction mixture was kept stirring in the ice bath for one hour, removed and stirred a further 2.5 hours at room temperature. The phosgene, EtOAc, and toluene were removed by vacuum distillation, leaving non-polydispersed compound 51 as a clear, oily residue.

The non-polydispersed residue **51** was dissolved in 20 mL dry dichloromethane and placed under a dry, inert atmosphere. Triethylamine (0.94 mL, 0.68 g, 6.7 mmol) and then NHS (N-hydroxy succinimide, 0.82 g, 7.1 mmol) were added, and the reaction mixture was stirred at room temperature for 18 hours. The mixture was filtered through silica gel to remove the white precipitate and concentrated *in vacuo*. The residue was taken up in dichloromethane and washed twice with cold water, twice with 1 N HCl and once with brine. The organics were dried over Na₂SO₄, filtered and concentrated. Final purification was done via flash chromatography (silica gel, EtOAc) to obtain the UV active non-polydispersed NHS ester **52**.

Example 41

Synthesis of Polypeptide-Oligomer Conjugates

Mixtures of polypeptide-oligomer conjugates according to the present invention are synthesized as follows. A mixture of the polypeptide is dissolved in anhydrous DMF. Then TEA and a mixture of an activated oligomer of Example 18, 24, 29, 31, 37, 38, 39 or 40 in anhydrous THF is added. The reaction mixture is then stirred, preferably for 1 hour. The reaction mixture is acidified (for example, by adding 2 mL of 0.1% TFA in water). The reaction is followed by HPLC. The reaction mixture is concentrated and purified by preparative liquid chromatography (for example, using Waters PrepLC™ 4000 RP Vydac C18 Protein and peptide, 1x25 column, water/acetonitrile with 0.1%TFA, detection at 280 nm). Peaks corresponding to mono- or multi-conjugated compounds are isolated. Samples may be analyzed by MALDI-MS.

Example 42

The procedure of Example 41 is performed and the polypeptide is an adrenocorticotrophic hormone peptide.

Example 43

The procedure of Example 41 is performed and the polypeptide is an adrenomedullin peptide.

Example 44

The procedure of Example 41 is performed and the polypeptide is an allatostatin peptide.

Example 45

The procedure of Example 41 is performed and the polypeptide is an amylin peptide.

Example 46

The procedure of Example 41 is performed and the polypeptide is an amyloid beta-protein fragment peptide.

Example 47

The procedure of Example 41 is performed and the polypeptide is an angiotensin peptide.

Example 48

The procedure of Example 41 is performed and the polypeptide is an antibiotic peptide.

Example 49

The procedure of Example 41 is performed and the polypeptide is an antigenic polypeptide.

Example 50

The procedure of Example 41 is performed and the polypeptide is an anti-microbial peptide.

Example 51

The procedure of Example 41 is performed and the polypeptide is an apoptosis related peptide.

Example 52

The procedure of Example 41 is performed and the polypeptide is an atrial natriuretic peptide.

Example 53

The procedure of Example 41 is performed and the polypeptide is a bag cell peptide.

Example 54

The procedure of Example 41 is performed and the polypeptide is a bombesin peptide.

Example 55

The procedure of Example 41 is performed and the polypeptide is a bone GLA peptide.

Example 56

The procedure of Example 41 is performed and the polypeptide is a bradykinin peptide.

Example 57

The procedure of Example 41 is performed and the polypeptide is a brain natriuretic peptide.

Example 58

The procedure of Example 41 is performed and the polypeptide is a C-peptide.

Example 59

The procedure of Example 41 is performed and the polypeptide is a C-type natriuretic peptide.

Example 60

150 mg of salmon calcitonin (MW 3432, 0.043 mmol) was dissolved in 30 ml of anhydrous DMF. Then TEA (35 μ L) and the activated oligomer of Example 24 (42 mg,

0.067 mmol) in anhydrous THF (2 mL) was added. The reaction was stirred for 1 hour, then acidified with 2 mL of 0.1% TFA in water. The reaction was followed by HPLC. Then the reaction mixture was concentrated and purified by preparative liquid chromatography (Waters PrepLC™ 4000 RC Vydac C18 Protein and peptide, 1x25 column, water/acetonitrile with 0.1% TFA, detection at 280 nm). Two peaks, corresponding to mono- and di-conjugate were isolated. Samples were analyzed by MALDI-MS. MS for PEG7-octyl-sCT, mono-conjugate: 3897. MS for PEG7-octyl-sCT, di-conjugate: 4361.

A similar procedure was used to conjugate salmon calcitonin with the activated oligomer of Example 29. MS for PEG7-decyl-sCT, mono-conjugate: 3926. MS for PEG7-decyl-sCT, di-conjugate: 4420.

A similar procedure was used to conjugate salmon calcitonin with the activated oligomer of Example 31. MS for stearate-PEG6-sCT, mono-conjugate: 4006. MS for stearate-PEG6-sCT, di-conjugate: 4582.

A similar procedure was used to conjugate salmon calcitonin with the activated oligomer of Example 37. MS for stearate-PEG8-sCT, mono-conjugate: 4095.

A similar procedure is used to conjugate salmon calcitonin with the activated oligomer of Example 18, 38, 39 and 40.

Example 61

The procedure of Example 41 is performed and the polypeptide is a calcitonin gene related peptide.

Example 62

The procedure of Example 41 is performed and the polypeptide is a CART peptide.

Example 63

The procedure of Example 41 is performed and the polypeptide is a casomorphin peptide.

Example 64

The procedure of Example 41 is performed and the polypeptide is a chemotactic peptide.

Example 65

The procedure of Example 41 is performed and the polypeptide is a cholecystokinin peptide.

Example 66

The procedure of Example 41 is performed and the polypeptide is a corticotropin releasing factor peptide.

Example 67

The procedure of Example 41 is performed and the polypeptide is a cortistatin peptide.

Example 68

The procedure of Example 41 is performed and the polypeptide is a dermorphin peptide.

Example 69

The procedure of Example 41 is performed and the polypeptide is a dynorphin peptide.

Example 70

The procedure of Example 41 is performed and the polypeptide is an endorphin peptide.

Example 71

The procedure of Example 41 is performed and the polypeptide is an endothelin peptide.

Example 72

The procedure of Example 41 is performed and the polypeptide is an ETa receptor antagonist peptide.

Example 73

The procedure of Example 41 is performed and the polypeptide is an ETb receptor antagonist peptide.

Example 74

The procedure of Example 41 is performed and the polypeptide is an enkephalin peptide.

Example 75

The procedure of Example 41 is performed and the polypeptide is a fibronectin peptide.

Example 76

The procedure of Example 41 is performed and the polypeptide is a galanin peptide.

Example 77

The procedure of Example 41 is performed and the polypeptide is a gastrin peptide.

Example 78

The procedure of Example 41 is performed and the polypeptide is a glucagon peptide.

Example 79

The procedure of Example 41 is performed and the polypeptide is a Gn-RH associated peptide.

Example 80

The procedure of Example 41 is performed and the polypeptide is a growth factor peptide.

Example 81

Human growth hormone was conjugated with the activated oligomers of Example 40

as illustrated in Figure 10. Human growth hormone (somatropin (tDNA origin) for injection), available under the trade name Saizen™ from Serono of Randolph, Massachusetts, was dissolved in DMSO such that the hGH was at a 0.58 mmol concentration. TEA (278 equivalents) was added and the solution was stirred for approximately ten minutes. Two equivalents, five equivalents or thirty equivalents of activated hexaethylene glycol 52 was added from a 0.2 M solution of the activated oligomer in dry THF. Reactions were stirred at room temperature for 45 minutes to one hour. Aliquots of each reaction mixture were quenched in 600 μ L of 0.1% TFA in water. HPLC comparison of the 2 polymer equivalent and the 5 polymer equivalent reaction mixtures vs. unconjugated hGH is shown in Figure 14. HPLC analysis of the thirty polymer equivalent reaction is shown in Figure 15.

Samples of the conjugates for mass spectroscopy were purified *via* analytical HPLC using a reversed-phase C₁₈ column and a water/acetonitrile gradient. The entire peak from the 2 equivalent reaction mixture was collected, concentrated and analyzed using MALDI mass spectroscopy. The mass spectra of this material showed evidence of the presence of mono-conjugated, di-conjugated, tri-conjugated and tetra-conjugated hGH as well as some remaining unreacted hGH (Figure 16). The five equivalent reaction mixture was purified crudely according to polarity as indicated in Figure 17. MALDI mass spectra of the concentrated fractions (Figure 18, Figure 19 and Figure 20) indicated that the level of conjugation of the protein increased with retention time. Electrospray mass spectra of fraction E, Figure 21, gave results consistent with the presence of hexa-conjugated hGH. The entire peak from the thirty polymer equivalent reaction mixture was collected and concentrated. Electrospray mass spectral analysis, Figure 22, showed deca- and higher conjugated material.

A similar procedure is used to conjugate hGH with the activated oligomer of Example 18, 24, 29, 31 or 37.

Example 82

Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated Palmitate-TEG Oligomers

Procedures similar to those described above in Example 81 were performed using the activated polymer from Example 39. Progress of conjugation was checked by HPLC by taking 20 μ L of the conjugated reaction mixture in a vial and diluting with 100 μ L of 0.1% TFA-water-IPA (1:1), the results of which are illustrated in Figure 23. After 2 hours, the

reaction was quenched by adding 0.1% TFA-water. The conjugated product was purified by prep. HPLC.

Example 83

Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated TEG Oligomers

One equivalent of human growth hormone (hGH) (somatropin (rDNA origin) for injection), available under the trade name Saizen™ from Serono of Randolph, Massachusetts was dissolved in DMSO (1 mg/125 μ L) and stirred at room temperature for 2-4 minutes. Two equivalents TEA was added followed by two equivalents of the activated oligomer of Example 38, which was dissolved in THF. After 2 hours, the reaction was quenched by adding 0.1% TFA-water. The conjugated product was purified by prep. HPLC as illustrated in Figure 24.

A similar procedure five equivalents TEA and five equivalents of the activated oligomer of Example 39 was performed. The conjugated product was purified by prep. HPLC using C18 column as illustrated in Figure 25. The mobile phase and elution time were as follows:

Time	mL/min	Solvent A	Solvent B
0	3.5	80	20
55	3.5	0	100

The pooled fraction was lyophilized into a white powder. The mass spectra for the compound are illustrated in Figures 26 and 27.

A similar procedure utilizing nine equivalents TEA and nine equivalents of the activated oligomer of Example 39 was performed. The conjugated product was purified by prep. HPLC using C18 column as illustrated in Figure 28.

Example 84

The procedure of Example 41 is performed and the polypeptide is a GTP-binding peptide.

Example 85

The procedure of Example 41 is performed and the polypeptide is a guanylin peptide.

Example 86

The procedure of Example 41 is performed and the polypeptide is an inhibin peptide.

Example 87**Synthesis of Insulin-Oligomer Conjugates**

To human insulin (zinc or zinc free, 2g, 0.344 mmol based on dry weight) in 25 mL dimethylsulfoxide (> 99% purity) at 22±4°C was added 8 mL triethyl amine (> 99% purity). The resulting mixture was stirred for 5 to 10 minutes at 22±4°C. To the above was rapidly added the activated oligomer of Example 18 above (0.188 g, 0.36 mmol based on 100% activation) in 7.5 mL acetonitrile under stirring at 22±4°C. The solution was stirred for 45 minutes and quenched with acetic acid solution with maintaining the temperature below 27°C. The reaction was monitored by analytical HPLC. This reaction condition produces PEG7-hexyl-insulin, monoconjugated at the B29 position (PEG7-hexyl-insulin, B29 monoconjugated) at yield 40-60%. The crude reaction mixture (PEG7-hexyl-insulin, B29 monoconjugated, 40-60%, unreacted insulin 8-25%, related substances 15-35%) was dialyzed or dialyzed (3000-3500 molecular weight cut off, MWCO) to remove organic solvents and small molecular weight impurities, exchanged against ammonium acetate buffer and lyophilized.

The conjugation reaction of PEG7-hexyl-insulin, monoconjugated at the B29 position, was monitored by analytical HPLC. This analytical HPLC method used a Waters Delta-Pak C18 column, 150 x 3.9 mm I.D., 5µm, 300 Å. The solvent system consisted of Solvent B: 0.1% TFA in 50/50 methanol/water, and Solvent D: 0.1% TFA in methanol. The gradient system was as follows:

Time (min)	% Solvent B	% Solvent D	Flow rate (mL/min)
Initial (0)	100	0	1.00
20	40	60	1.00
25	100	0	1.00

A similar procedure is used to provide non-polydispersed mixtures of insulin-oligomer conjugates using the activated oligomers of Example 24, 29, 31, 37, 38, 39 and 40.

Example 88

The procedure of Example 41 is performed and the polypeptide is an interleukin peptide.

Example 89

The procedure of Example 41 is performed and the polypeptide is a leptin peptide.

Example 90

The procedure of Example 41 is performed and the polypeptide is a leucokinin peptide.

Example 91

The procedure of Example 41 is performed and the polypeptide is a luteinizing hormone-releasing hormone.

Example 92

The procedure of Example 41 is performed and the polypeptide is a mastoparan peptide.

Example 93

The procedure of Example 41 is performed and the polypeptide is a mast cell degranulating peptide.

Example 94

The procedure of Example 41 is performed and the polypeptide is a melanocyte stimulating hormone peptide.

Example 95

The procedure of Example 41 is performed and the polypeptide is a morphiceptin peptide.

Example 96

The procedure of Example 41 is performed and the polypeptide is a molin peptide.

Example 97

The procedure of Example 41 is performed and the polypeptide is a neuro-peptide.

Example 98

The procedure of Example 41 is performed and the polypeptide is a neuropeptide Y peptide.

Example 99

The procedure of Example 41 is performed and the polypeptide is a neurotropic factor peptide.

Example 100

The procedure of Example 41 is performed and the polypeptide is an oxnin peptide.

Example 101

The procedure of Example 41 is performed and the polypeptide is an opioid peptide.

Example 102

The procedure of Example 41 is performed and the polypeptide is an oxytocin peptide.

Example 103

The procedure of Example 41 is performed and the polypeptide is a PACAP peptide.

Example 104

The procedure of Example 41 is performed and the polypeptide is a paeceastatin peptide.

Example 105

The procedure of Example 41 is performed and the polypeptide is a pancreatic polypeptide.

Example 106

The procedure of Example 41 is performed and the polypeptide is a parathyroid hormone peptide.

Example 107

The procedure of Example 41 is performed and the polypeptide is a parathyroid hormone-related peptide.

Example 108

The procedure of Example 41 is performed and the polypeptide is a peptide T peptide.

Example 109

The procedure of Example 41 is performed and the polypeptide is a prolactin-releasing peptide.

Example 110

The procedure of Example 41 is performed and the polypeptide is a peptide YY peptide.

Example 111

The procedure of Example 41 is performed and the polypeptide is a renin substrate peptide.

Example 112

The procedure of Example 41 is performed and the polypeptide is a secretin peptide.

Example 113

The procedure of Example 41 is performed and the polypeptide is a somatostatin

peptide.

Example 114

The procedure of Example 41 is performed and the polypeptide is a substance P peptide.

Example 115

The procedure of Example 41 is performed and the polypeptide is a tachykinin peptide.

Example 116

The procedure of Example 41 is performed and the polypeptide is a thyrotropin-releasing hormone peptide.

Example 117

The procedure of Example 41 is performed and the polypeptide is a toxin peptide.

Example 118

The procedure of Example 41 is performed and the polypeptide is a vasoactive intestinal peptide.

Example 119

The procedure of Example 41 is performed and the polypeptide is a vasopressin peptide.

Example 120

The procedure of Example 41 is performed and the polypeptide is a virus related peptide.

Example 121

**Purification of B29 Modified PEG7-Hexyl-Insulin,
Monocoujugate, from the Crude Mixture**

PEG7-hexyl-insulin, B29 monocoujugated, was purified from the crude mixture of

Example 87 using a preparative HPLC system. Lyophilized crude mixture (0.5 g, composition: PEG7-hexyl-insulin, B29 monoconjugate, 40-60%, unreacted insulin 8-25%, related substances 15-35%) was dissolved in 5-10 mL 0.01 M ammonium acetate buffer, pH 7.4 and loaded to a C-18 reverse phase HPLC column (150 x 3.9 mm) equilibrated with 0.5% triethylamine/0.5% phosphoric acid buffer TEAP A). The column was eluted with a gradient flow using TEAP A and TEAP B (80% acetonitrile and 20% TEAP A) solvent system. The gradient system for preparative HPLC purification of PEG7-hexyl-insulin, B29 monoconjugate, from the crude mixture was as follows:

Time (min)	% TEAP A	% TEAP B	Flow rate (mL/min)
Initial (0)	70	30	30
45	64	36	30
105	60	40	30
115	40	60	30
125	15	85	30
135	15	85	30

Fractions were analyzed by HPLC and the product fractions that were >97% purity of PEG7-hexyl-insulin, B29 monoconjugate, were pooled. The elution buffer and solvent were removed by dialysis or diafiltration (MWCO 3000-3500) against ammonium acetate buffer (0.01 M, pH 7.4) and exchanged into ammonium acetate buffer and lyophilized to produce white powder of PEG7-hexyl-insulin, B29 monoconjugate (purity >97%).

An analytical HPLC method using the same column and solvent system as the method used in Example 87 to monitor the reaction was used for analysis of PEG7-hexyl-insulin, B29 monoconjugate. However, the gradient conditions were as follows:

Time (min)	% Solvent B	% Solvent D	Flow rate (mL/min)
Initial (0)	100	0	1.00
30	10	90	1.00
35	100	0	1.00

Example 122**Determination of the Dispersity Coefficient
for a Mixture of Human Insulin-Oligomer Conjugates**

The dispersity coefficient of a mixture of human insulin-oligomer conjugates is determined as follows. A mixture of human insulin-oligomer conjugates is provided, for example as described above in Example 97. A first sample of the mixture is purified via HPLC to separate and isolate the various human insulin-oligomer conjugates in the sample. Assuming that each isolated fraction contains a purely monodispersed mixture of conjugates, "n" is equal to the number of fractions collected. The mixture may include one or more of the following conjugates, which are described by stating the conjugation position followed by the degree of conjugation: Gly^{A1} monoconjugate; Phe^{B1} monoconjugate; Lys^{B29} monoconjugate; Gly^{A1}, Phe^{B1} diconjugate; Gly^{A1}, Lys^{B29} diconjugate; Phe^{B1}, Lys^{B29} diconjugate; and/or Gly^{A1}, Phe^{B1}, Lys^{B29} triconjugate. Each isolated fraction of the mixture is analyzed via mass spectroscopy to determine the mass of the fraction, which allows each isolated fraction to be categorized as a mono-, di-, or tri-conjugate and provides a value for the variable "M_i" for each conjugate in the sample.

A second sample of the mixture is analyzed via HPLC to provide an HPLC trace. Assuming that the molar absorptivity does not change as a result of the conjugation, the weight percent of a particular conjugate in the mixture is provided by the area under the peak of the HPLC trace corresponding to the particular conjugate as a percentage of the total area under all peaks of the HPLC trace. The sample is collected and lyophilized to dryness to determine the anhydrous gram weight of the sample. The gram weight of the sample is multiplied by the weight percent of each component in the sample to determine the gram weight of each conjugate in the sample. The variable "N_i" is determined for a particular conjugate (the ith conjugate) by dividing the gram weight of the particular conjugate in the sample by the mass of the particular conjugate and multiplying the quotient by Avogadro's number (6.02205×10^{23} mole⁻¹), M₀, determined above, to give the number of molecules of the particular conjugate, N_i, in the sample. The dispersity coefficient is then calculated using n, M_i as determined for each conjugate, and N_i as determined for each conjugate.

Example 123**Cytosensor Studies for Insulin-Oligomer Conjugates**

Colo 205 (colorectal adenocarcinoma cells from ATCC, catalog #CCL-222) cells that had been serum-deprived for approximately 18 hours were suspended in 3:1 Cytosensor low-buffer RPMI-1640 media: Cytosensor agarose entrapment media and seeded into Cytosensor capsule cups at 100,000 cells/10 μ L droplets. Cells were allowed to equilibrate on the Cytosensor to the low-buffer RPMI-1640 media at a flow rate of 100 μ L per minute for approximately 3 hours until baseline acidification rates were stable. Insulin drugs (insulin or insulin conjugates) were diluted to 50nM in low-buffer RPMI-1640 media and applied to the cells for 20 minutes at 100 μ L/minute. Following the exposure, the drug solutions were withdrawn and the cells were again perfused under the continuous flow of low-buffer media alone. Data collection continued until acidification rates returned to baseline levels (approximately one hour from application of drug solutions). The results are illustrated in Figure 29. As used in Figure 29, insulin is human insulin; PEG4 is a non-polydispersed mixture of mPEG4-hexyl-insulin, B29 monoconjugates; PEG10 is a non-polydispersed mixture of mPEG10-hexyl-insulin, B29 monoconjugates; PEG7 is a non-polydispersed mixture of mPEG7-hexyl-insulin, B29 monoconjugates; PEG7_{avg} is a polydispersed mixture of mPEG7_{avg}-hexyl-insulin, B29 monoconjugates.

Example 124**Enzymatic Stability of Insulin-Oligomer Conjugates**

Chymotrypsin digests were conducted in a phosphate buffer, pH 7.4, at 37°C in a shaking water bath. The insulin/insulin conjugate concentration was 0.3 mg/mL. The chymotrypsin concentration was 2Units/mL. 100 μ L samples were removed at the indicated time points and quenched with 25 μ L of a 1:1 mixture of 0.1% trifluoroacetic acid/isopropyl alcohol. Samples were analyzed by reverse phase HPLC and the relative concentrations of insulin/insulin conjugate were determined by calculating the areas under the curves.

As used in Figure 30, insulin is human insulin; PEG4 is a non-polydispersed mixture of mPEG4-hexyl-insulin, B29 monoconjugates; PEG10 is a non-polydispersed mixture of mPEG10-hexyl-insulin, B29 monoconjugates; PEG7 is a non-polydispersed mixture of

mPEG7-hexyl-insulin, B29 monoconjugates; PEG7_{AVG} is a polydispersed mixture of mPEG7_{AVG}-hexyl-insulin, B29 monoconjugates.

Example 125

Dose Dependent Activity for Insulin-Oligomer Conjugates

An effective animal model for evaluating formulations uses normal fasted beagle dogs. These dogs are given from 0.25 mg/kg to 1.0 mg/kg of insulin conjugates to evaluate the efficacy of various formulations. This model was used to demonstrate that insulin conjugates according to the present invention provide lower glucose levels in a dose dependent manner better than polydispersed insulin conjugates, which are not part of the present invention and are provided for comparison purposes.

The protocol for dog experiments calls for a blood glucose measurement at time zero just before a drug is administered. The formulation in solid oral dosage form is then inserted into the dog's mouth. Blood is drawn at 15, 30, 60 and 120 minutes and glucose levels are measured and graphed. The lower the glucose levels, the better the activity of the insulin conjugate. In Figure 31, the glucose lowering, and thus the activity, of the conjugates of the present invention is shown to be dose dependent. For comparison purposes, Figure 32 shows that the glucose lowering of polydispersed insulin conjugates in a capsule formulation, which are not a part of the present invention, is less dose dependent than conjugates of the present invention.

Example 126

Activity and Inter-Subject Variability for Insulin-Oligomer Conjugates

An effective animal model for evaluating formulations uses normal fasted beagle dogs. These dogs are given 0.25 mg/kg of insulin conjugates to evaluate the efficacy of various formulations. This model was used to demonstrate that insulin conjugates according to the present invention provide lower inter-subject variability and better activity than polydispersed insulin conjugates, which are not part of the present invention but are provided for comparison purposes.

The protocol for dog experiments calls for a blood glucose measurement at time zero just before a drug is administered. The oral liquid dosage formulation is then squirted into the back of the dog's mouth. In each case, the dogs received 0.25 mg/kg of this solution.

Blood is drawn at 15, 30, 60 and 120 minutes and glucose levels are measured and graphed. The lower the glucose levels, the better the activity of the insulin conjugate. In Figures 33, 34 and 35, the results obtained with PEG4-hexyl-insulin, monoconjugate; PEG7-hexyl-insulin, monoconjugate; and PEG10-hexyl-insulin, monoconjugate, respectively, show these PEG conjugates of the present invention result in less inter-subject variability and higher activity than the results shown in Figure 36 for the polydispersed PEG7_{avg}-hexyl-insulin, monoconjugate, which is not a part of the present invention and is provided for comparison purposes.

Example 127

Cytosensor® Studies for Calcitonin-Oligomer Conjugates

T-47D cells (mammary ductal carcinoma cell line, obtained from American Type Culture Collection) were suspended at a density of 1×10^5 cells/mL in running buffer (low-buffered, serum-free, bicarbonate-free RPMI 1640 medium from Molecular Devices of Sunnyvale, California). Approximately 100,000 cells were then immobilized in an agarose cell entrapment medium in a 10 μ L droplet and sandwiched between two 3- μ m polycarbonate membranes in a cytosensor capsule cup. Cytosensor capsule cups placed in sensor chambers on the Cytosensor® Microphysiometer were then held in very close proximity to pH-sensitive detectors. Running buffer was then pumped across the cells at a rate of 100 μ L/min except during 30-second intervals when the flow was stopped, and acidification of the running buffer in the sensor chamber was measured. Acidification rates were determined every 2 minutes. The temperature of the sensor chambers was 37°C. Cells were allowed to equilibrate in the sensor chambers for 2-3 hours prior to the start of the experiment during which time basal acidification rates were monitored. Cells were then exposed to test compounds (Salmon Calcitonin or Octyl-Di-Calcitonin) diluted in running buffer at various nM concentration. Exposure of cells to test compounds occurred for the first 40 seconds of each 2 minute pump cycle in a repeating pattern for a total of 20 minutes. This allowed sufficient exposure of the cells to the test compounds to elicit a receptor-mediated response in cellular metabolism followed by approximately 50 seconds of flow of the running buffer containing no compounds. This procedure rinsed away test solutions (which had a slightly lower pH than running buffer alone) from the sensor chamber before measuring the acidification rate. Thus, the acidification rates were solely a measure of cellular activity. A similar procedure was

used to obtain data for PEG7-octyl-sCT, monoconjugate (Octyl-Mono); PEG7-decyl-sCT, monoconjugate (Decyl-Mono); PEG7-decyl-sCT, diconjugate (Decyl-Di); stearate-PEG6-sCT, monoconjugate (PEG6 St. Mono); and stearate-PEG8-sCT, monoconjugate (PEG8 St. Mono). Data was analyzed for relative activity of compounds by calculating the Area Under the Curve (AUC) for each cytosensor chamber acidification rate graph and plotted as a bar chart illustrated in Figure 37 showing average AUC measurements taken from multiple experiments performed under the same experimental conditions.

Example 128

Enzymatic Stability for Calcitonin-Oligomer Conjugates

Compounds, supplied as lyophilized powders, are resuspended in 10 mM phosphate buffer pH 7.4 and then submitted for concentration determination by HPLC. The phosphate buffer is used to create a solution with a pH that is optimum for activity of each particular gut enzyme. Aliquots of the compound thus prepared are transferred to 1.7 mL microcentrifuge tubes and shaken in a 37°C water bath for 15 minutes to allow compounds to equilibrate to temperature. After 15 minutes, 2 μ L of the appropriate concentrated gut enzyme is added to each tube to achieve the final concentration desired. Chymotrypsin and trypsin are resuspended in 1 mM HCl. Also, as a control, compounds are treated with 2 μ L of 1 mM HCl. Immediately following additions, 100 μ L of sample is removed from the control tube and quenched with either 25 μ L of chymotrypsin/trypsin quenching solution (1:1 1% TFA:Isopropanol). This sample will serve as T=0 min. A sampling procedure is repeated at various time intervals depending on the gut enzyme used. Chymotrypsin has 15, 30 and 60 minute samples. Trypsin has 30, 60, 120 and 180 minute samples. Once all points have been acquired, a final sample is removed from the control tube to make sure that observed degradation is not temperature or buffer related. The chymotrypsin and trypsin samples may be collected directly into HPLC vials. RP-HPLC (acetonitrile gradient) is used to determine AUC for each sample and % degradation is calculated based from the T=0 min control. The results are provided below in Tables 1 to 4.

Table 1

% Remaining Following 0.5 U/mL Chymotrypsin Digest
of PEG7-Octyl-Salmon Calcitonin, Diconjugate

Time	Non-Formulated					Buffered Formulation		
15	63	71	68	69		88	86	88
30	34	48	50	46		73	88	86
60	6	15	20	15		61	69	84
	Control					Control		
60	104	88	97	103		116	104	101

Table 2

% Remaining Following 0.5 U/mL Chymotrypsin Digest of Salmon Calcitonin
(for comparison purposes; not part of the invention)

Time	Non-Formulated					Buffered Formulation		
10	73							
15	-	55	62	35	66	59	91	92
30	30	26	40	13	42	54	86	87
60	1.6	5	12	1	12	55	82	85
	Control					Control		
60	-	100	93	45	100	102	98	103

Table 3

% Remaining following 1 U/mL Trypsin Digest
of PEG7-Octyl-Salmon Calcitonin, Disconjugate

Time	Non-Formulated			
30	87	89	83	90
60	78	86	76	85
120	72	82	68	78
180	-	81	61	73
Control				
60		103	100	
120	106	105	99	
180		104	99	

Table 4

% Remaining following 1 U/mL Trypsin Digest of Salmon Calcitonin
(for comparison purposes; not part of the invention)

Time	Non-Formulated			
30	80	50	82	87
60	66	28	69	76
120	44	7	46	59
180	-	2	31	46
Control				
60		41	101	
120	69	16	102	
180		7	101	

Example 130**Activity and Inter-Subject Variability for Calcitonin-Oligomer Conjugates**

Male CF-1 mice (Charles River, Raleigh, NC) weighing 20-25 g were housed in the Nobex vivarium in a light- (L:D cycle of 12:12, lights on at 0600 h), temperature- (21-23°C), and humidity- (40-60 % relative humidity) controlled room. Animals were permitted free access to laboratory chow (PMI Nutrition) and tap water. Mice were allowed to acclimate to housing conditions for 48-72 hours prior to the day of experiment.

Prior to dosing, mice were fasted overnight and water was provided ad libitum. Mice were randomly distributed into groups of five animals per time point and were administered a single oral dose of a PEG7-octyl-sCT, diconjugate (Octyl Di) according to the present invention or salmon calcitonin (sCT or Calcitonin) for comparison purposes. Oral doses were administered using a gavage needle (Popper #18, 5 cm from hub to bevel) at 10 mL/kg in the following 0.2 µg/mL phosphate-buffered PEG7-octyl-sCT, diconjugate, formulation:

Ingredient	Amount
PEG7-octyl-sCT, diconjugate	20 µg
Sodium-cholate	2.5 g
Sodium-deoxy-cholate	2.5 g
Sodium phosphate buffer, 100 mM, pH 7.4	q.s. to 100 g

The buffered formulation was prepared by adding 80 mL of phosphate buffer in a clean tared glass beaker. The sodium cholate was slowly added to the phosphate buffer with stirring until dissolved. The deoxy cholate was then added and stirring was continued until dissolved. The PEG7-octyl-sCT, diconjugate, solution equivalent to 20 µg was added. Finally, the remaining phosphate buffer was added to achieve a final weight of 100 g. Vehicle-control mice were used in all experiments. Dose-response curves were constructed using a single time point 60 minutes after drug administration. These curves are illustrated in Figures 38-41.

At appropriate time points, mice were ether-anesthetized, the vena caeve exteriorized, and blood samples were obtained via a syringe fitted with a 25-gauge needle. Blood aliquots were allowed to clot at 22°C for 1 hour, and the sera removed and pipetted into a clean receptacle. Total serum calcium was determined for each animal using a calibrated Vitros DT60 II analyzer.

Serum calcium data were plotted and pharmacokinetic parameters determined via

curve-fitting techniques using SigmaPlot software (Version 4.1). Means and standard deviations (or standard errors) were calculated and plotted to determine effect differences among dosing groups. Average serum calcium data for various conjugates are provided in Table 5 below.

Table 5

Conjugate	Dispersity	% Baseline Calcium Drop at 2.0 µg/kg dose
PEG7-Octyl-sCT, diconjugate	Monodispersed mixture	21.0
Stearate-PEG6-sCT, diconjugate	Monodispersed mixture	16.0
PEG7-Decyl-sCT, monoconjugate	Monodispersed mixture	11.5
Stearate-PEG8-sCT, diconjugate	Monodispersed mixture	11.0
PEG7-Decyl-sCT, diconjugate	Monodispersed mixture	8.3

Despite an *in vitro* activity as determined in Example 50 above that may not be comparable with the *in vitro* activity of PEG7-octyl-sCT and PEG7-decyl-sCT mono- and di-conjugates, the stearate-PEG6-sCT, diconjugate, and stearate-PEG8-sCT, diconjugate, appear to have *in vivo* activity (as evidenced by the drops in % baseline calcium from Table 5 above) that are comparable with the *in vivo* activity observed for the PEG7-octyl-sCT and PEG7-decyl-sCT, mono- and di-conjugates. While not wanting to be bound by a particular theory, the improved *in vivo* activity of the stearate containing conjugates may indicate that these conjugates are undergoing hydrolysis *in vivo* to provide an active salmon calcitonin or active salmon calcitonin-PEG conjugate.

Example 131

The assay is as follows:

Cell culture: Stable clones expressing the full length human GHR were generated in 293 cells (human kidney embryonal cell line), designated 293GHR, as previously described.

Transcription assays: These were performed in 293 GHR cells transiently transfected with a reported construct containing a Stat5-binding element (LHRE) fused to a minimal TK promoter and luciferase. A β -galactosidase expression vector was cotransfected as a transfection control and luciferase values corrected for β -galactosidase activity. Sixteen hours after transfection, cells were transferred into serum free medium and treated with GH

or agonist for 6 hours. Luciferase activity is reported as percentage of maximal activity stimulated by GH in the specific experiment to allow comparison between repeated experiments. The maximal activity stimulated by GH is the fold induction stimulated by GH, i.e. corrected luciferase value in GH stimulated cells divided by corrected luciferase value in unstimulated cells. Results of the assay are shown in Figures 42 and 43 where Genotropin is human growth hormone (standard, not part of the present invention), GH-002 is a 2 equivalent mTEG conjugate, GH-003 is a 5 equivalent mTEG conjugate, GH-004 is a 5 equivalent mTEG conjugate, Prot hGH is human growth hormone (standard, not part of the present invention), and hGH-TEG is a 9 equivalent mTEG conjugate.

In the specification, there has been disclosed typical preferred embodiments of the invention and, although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation, the scope of the invention being set forth in the following claims.

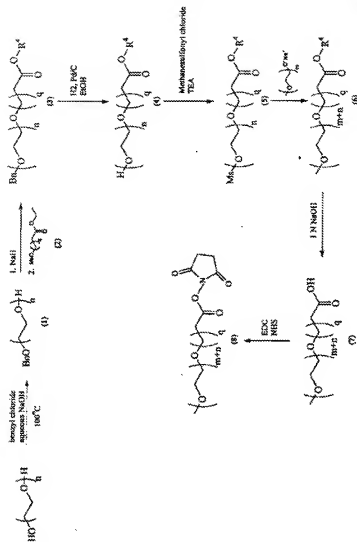


Figure 1

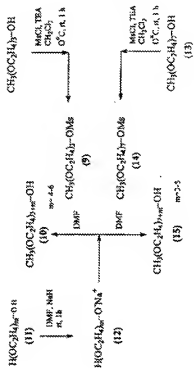


Figure 2

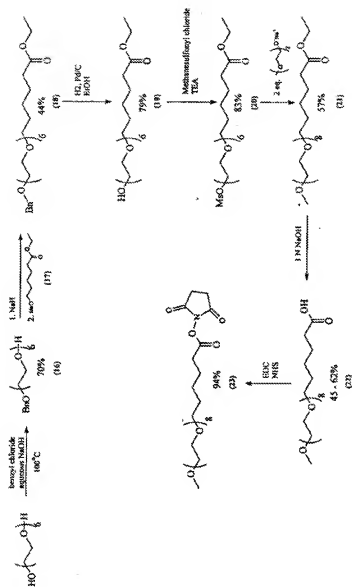


Figure 3

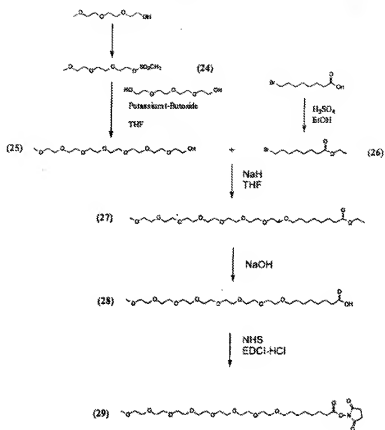


Figure 4

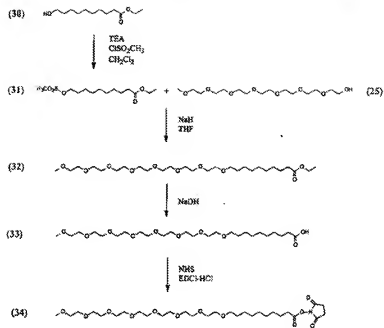


Figure 5

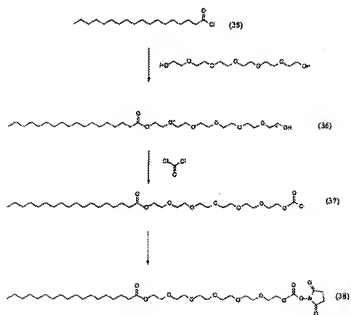


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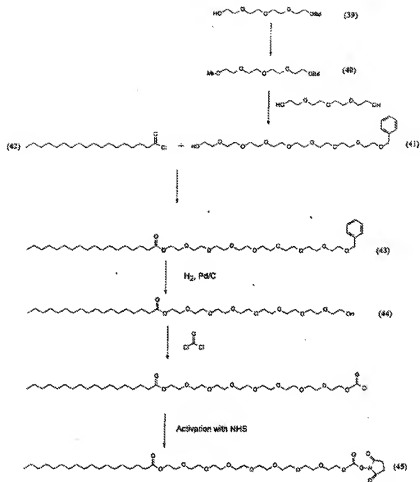


Figure 7

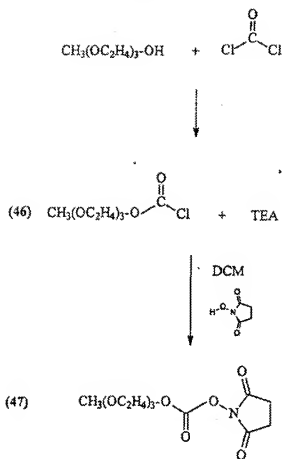


Figure 8

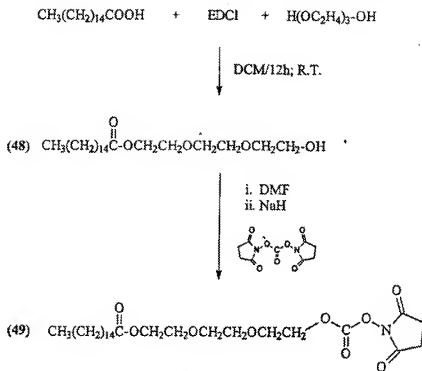


Figure 9

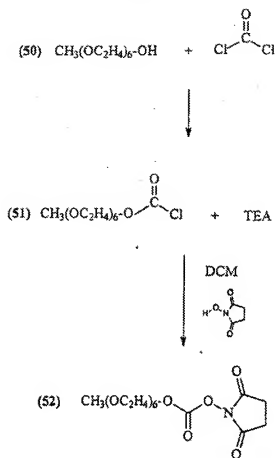


Figure 10

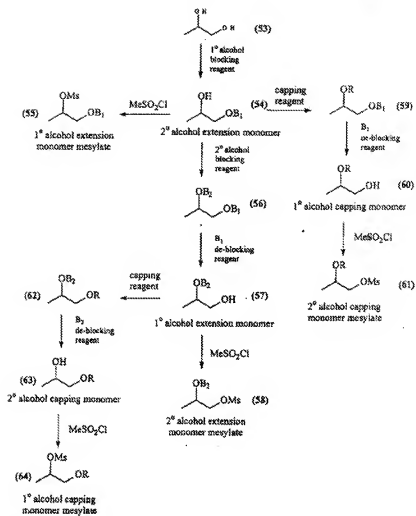


Figure 11

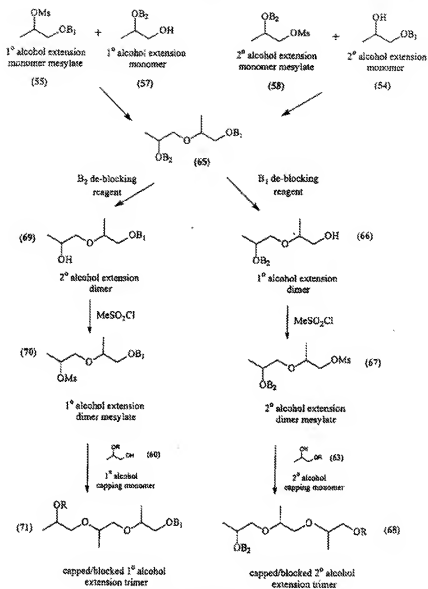


Figure 12

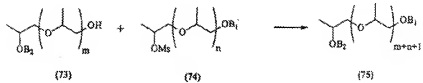


Figure 13

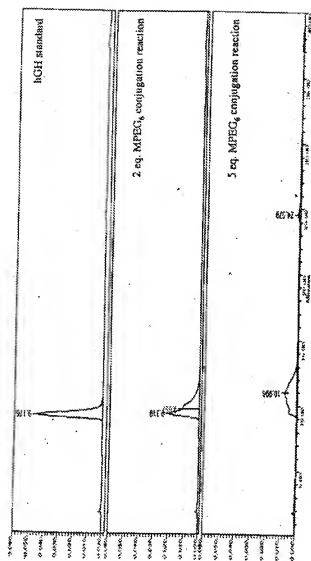


Figure 14

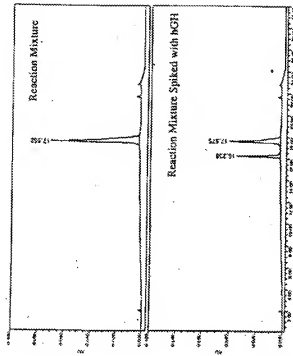


Figure 15

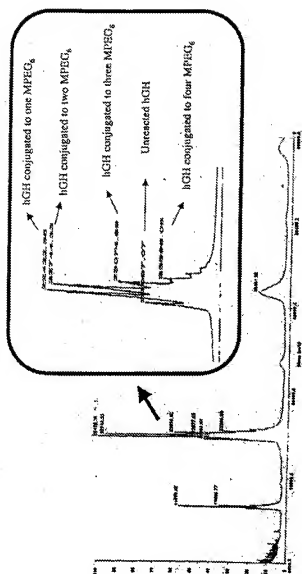


Figure 16

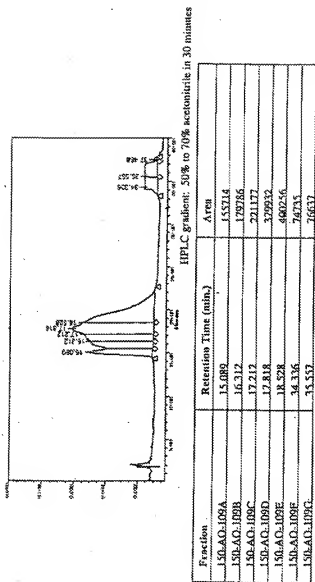


Figure 17

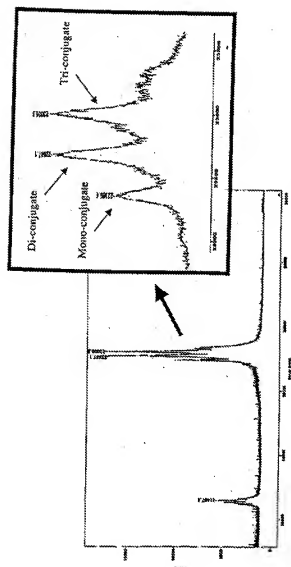


Figure 18

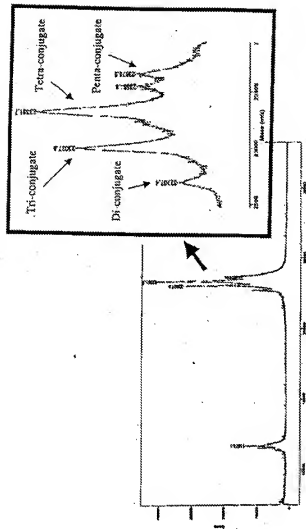


Figure 19

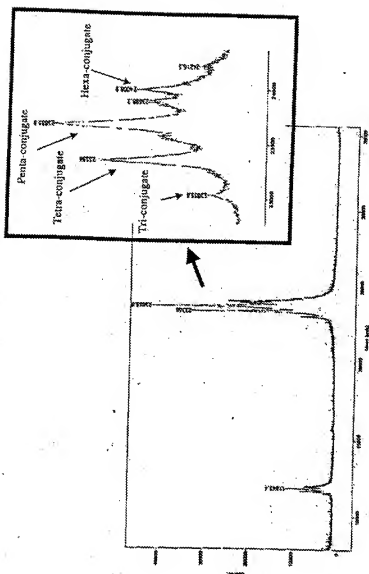


Figure 20

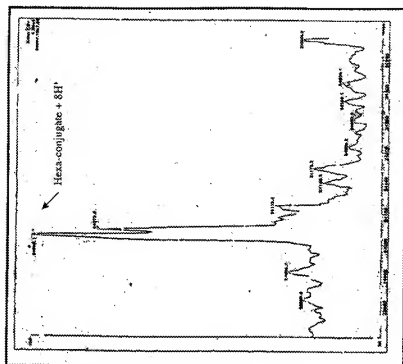


Figure 21

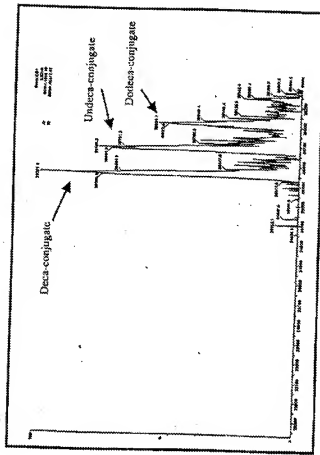


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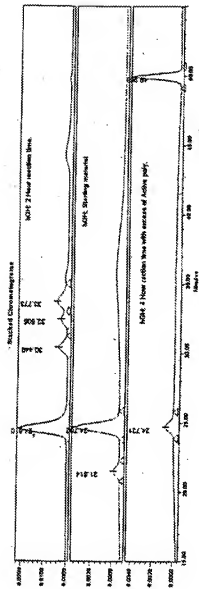


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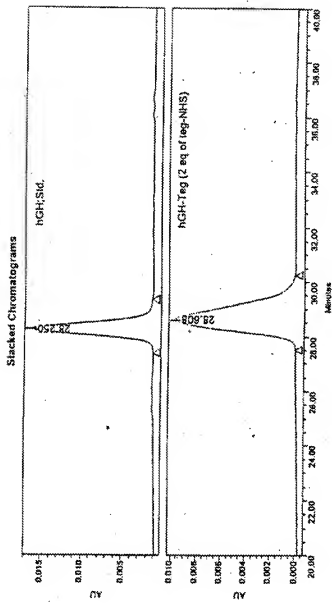


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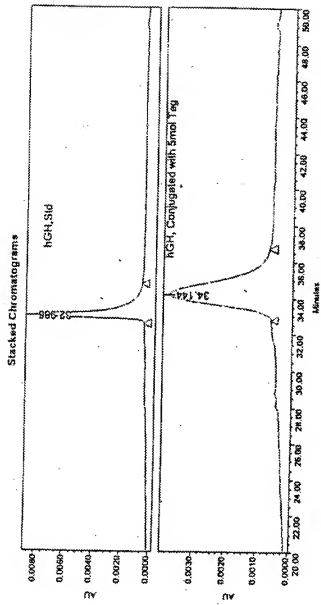


Figure 25

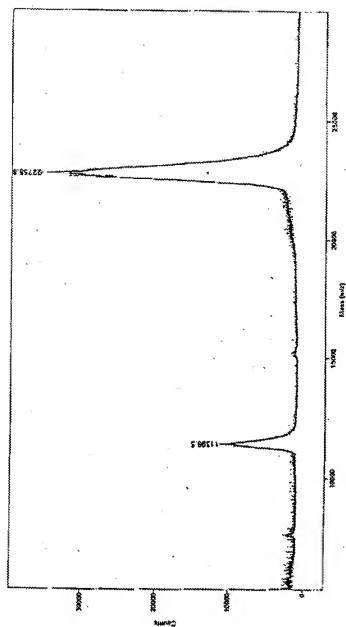


Figure 26

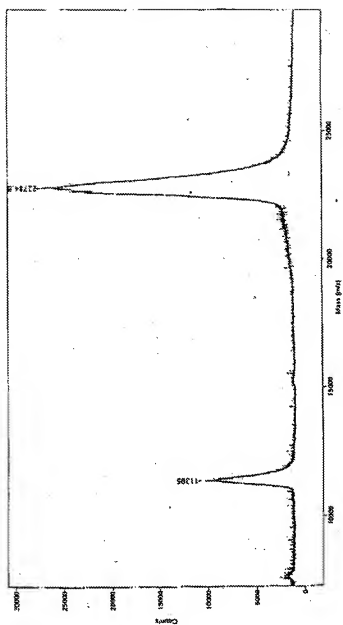


Figure 27

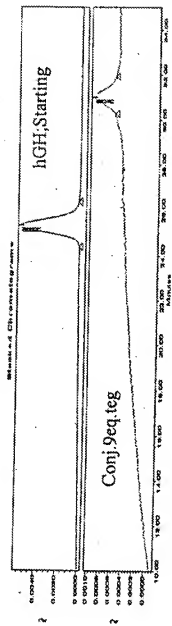


Figure 28

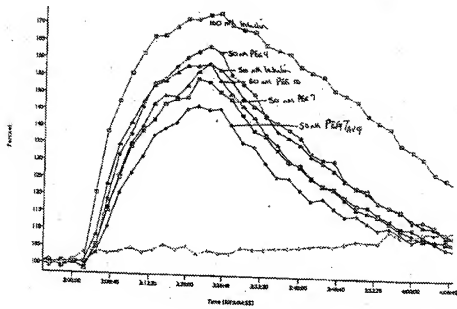


Figure 29

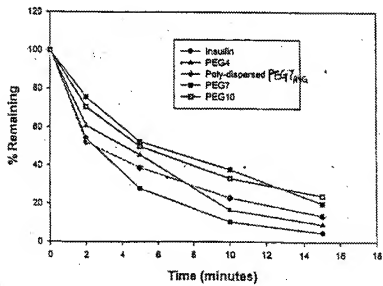
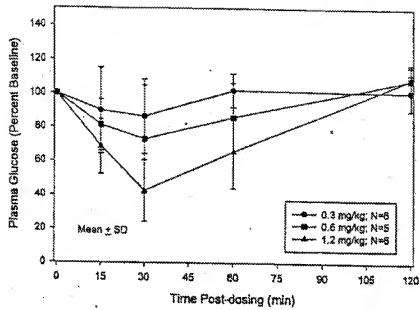


Figure 30

**Figure 31**

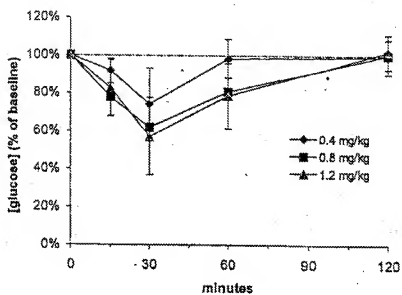


Figure 32

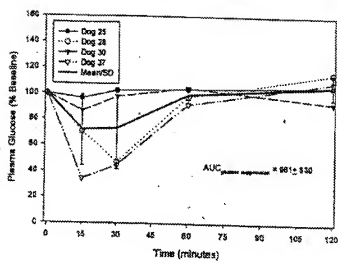


Figure 33

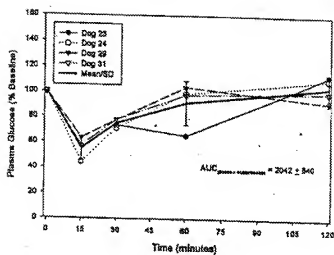


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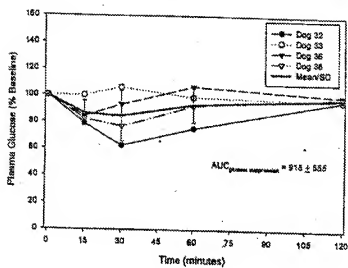


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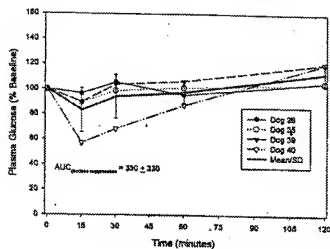
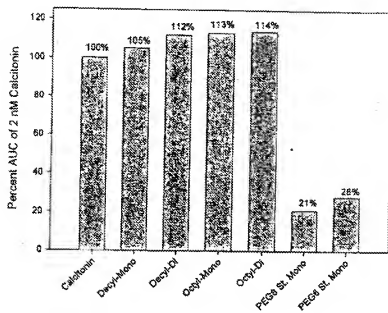
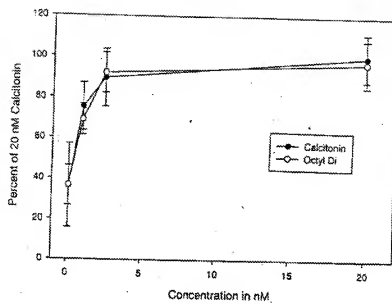
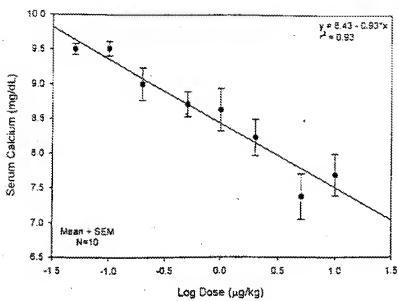


Figure 36

**Figure 37**

**Figure 38**

**Figure 39**

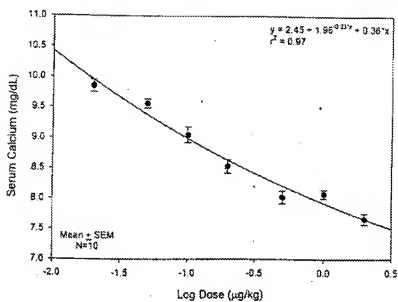


Figure 40

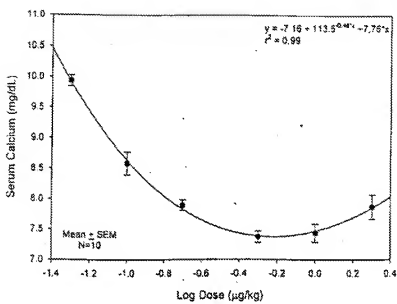


Figure 41

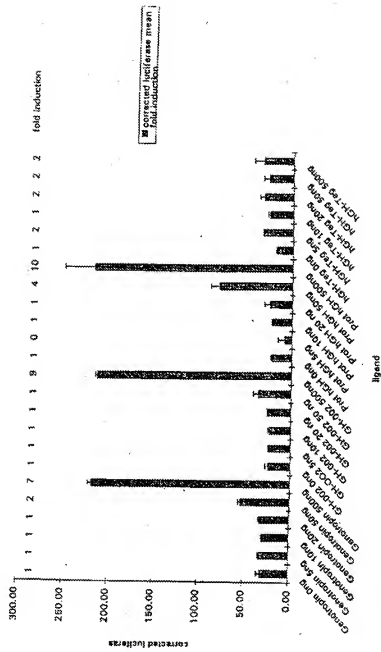


Figure 42

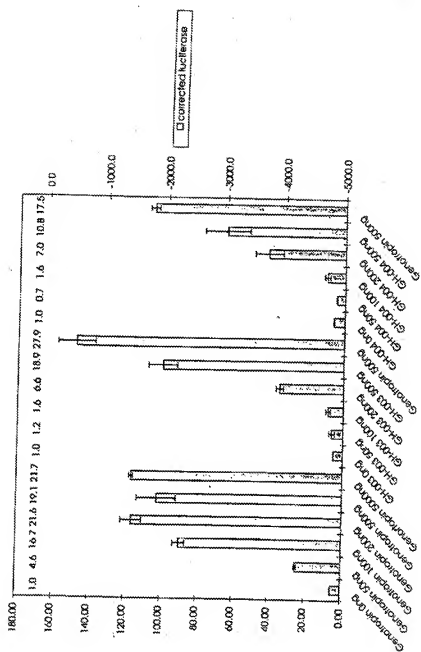


Figure 43

Abstract**Abstract Of The Disclosure**

A non-polydispersed mixture of conjugates in which each conjugate in the mixture comprises a drug coupled to an oligomer that includes a polyalkylene glycol moiety is disclosed. The mixture may exhibit higher *in vivo* activity than a polydispersed mixture of similar conjugates. The mixture may be more effective at surviving an *in vitro* model of intestinal digestion than polydispersed mixtures of similar conjugates. The mixture may result in less inter-subject variability than polydispersed mixtures of similar conjugates.